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A one ml ampule of frozen D-234 stock (ATCC HB-9543) was thawed quickly in a 37°C water bath. The contents were aseptically added to 100 ml prewarmed, pregassed (95% air and 5% CO₂), serum-free HL-1 medium (Ventrex Labs, Portland, [Me]ME) supplemented with 0.1% Pluronic polyol F-68 and 8 mM L-glutamine in a 250 ml Erlenmyer flask with a loosely fitted plastic screw cap. The flask was placed in a humidified incubator (36.5°C, 90% relative humidity and 5% CO₂) and cultured with shaking at 100-120 revolutions per minute (rpm).

In the Claims:

cancel

Please cancel claim 2 without prejudice to or disclaimer of the subject matter encompassed thereby.

Please amend claims 1, 3, and 6 to read as follows:

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cancel

1. (amended) A method of determining the optimal level of product expression in animal cell culture wherein the concentration of a solute of interest in a culture medium composition for optimal product expression is different than the concentration of said solute in the culture medium composition determined for optimal cell growth, which method comprises:

- a) growing the animal cell culture in a culture medium to determine optimal cell growth;
- b) varying the concentration of the solute in the culture medium to a concentration above that optimal for cell growth, which concentration is effective to create an environment of solute stress on the cell culture as expressed by an inhibitory effect on cell growth or cell density of said cell culture;
- c) monitoring the product expression as concentration of the solute is varied in the culture medium to determine optimal product expression; and
- d) selecting the solute concentration that provides the optimal combination of cell growth and product expression, which allows for optimal productivity.